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Serum renotropic activity and renal growth in spontaneously hypertensive rats

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In the 1950's Braun Menendez [1, 2] noted that the production of experimental hypertension often involved reduction in renal mass. Accordingly, he proposed that a substance, probably an intermediary product of protein metabolism responsible for renal growth (renotropin), led to hypertension as a secondary event: Hypertension develops when the remaining renal tissue is unable to respond to the growth stimulator, which, in turn, creates increased concentrations of renotropin. The weakness in this proposal was that no assay had been developed to prove the existence of a specific circulating renal growth factor.

Since then, experimental evidence suggests that a circulating substance(s) that incites and/or regulates compensatory renal growth (CRG) (hypertrophy, hyperplasia) in response to decreased renal mass does exist. Through parabiosis [3–10], in vivo injections of serum or plasma [11–13] and in vitro incubations [14–21], studies from many laboratories have demonstrated a renotropic factor affecting various aspects of growth. The in vitro studies show that plasma and serum from rats 12 to 48 hr after unilateral nephrectomy enhance the incorporation of radiolabelled nuclides into RNA [14, 15, 19] and DNA [14, 16–18, 20] and even increase the protein and dry weight of incubating renal slices [21].

Because viable assays currently exist to demonstrate renotropic activity, at least in plasma and serum removed from unilaterally nephrectomized rats [14, 21], we wished to discern whether or not there is enhanced circulating renotropic activity in hypertensive rats. We chose to compare the renotropic activity of serum from spontaneously hypertensive rats (SHR) with that from various strains of normotensive rats. In addition, some characteristics of CRG in SHR were assessed. We found a relative augmentation in circulating renotropic activity despite a lack of evidence of excess renal growth in young SHR.

Methods

All rats were housed in a well ventilated room maintained at 22°C with a light/dark phase of 14 and 10 hr. Three to four rats were kept together in plastic cages. Spontaneously hypertensive rats (SHR), Wistar Kyoto rats (WKY), and Sprague-Dawley rats (SD) were all obtained from Taconic Farms, Germantown, New York. The normotensive American Wistar rats (NWR) were purchased from Microbiological Associates, Walkersville, Maryland. All rats were obtained at least 1 week prior to being studied. Different aged rats were used in the different studies as indicated in the text, but all rats were under 25 weeks of age. Like others, we have found consistently that the SHR rats of greater than 10 weeks of age develop a blood pressure exceeding 150 mm Hg [22].

Sham operations and unilateral nephrectomy were performed as previously described [14]. In the studies depicted in Table 1, the animals were killed at specified times following the operative procedures. Sera and renal extracts were obtained from unilaterally nephrectomized and sham-operated rats 18 to 20 hr postoperation. The in vitro assays for renotropic activity were performed on renal fragments obtained from control Sprague-Dawley rats [23]. The assay for renotropic activity has been described previously in detail [16, 17]. We obtained renal extracts from the kidneys of the rats in the same manner as previously described [17]. Two different types of investigations were performed. In the first, we utilized a system in which a combination of renal extracts and serum from NWR and SHR rats were investigated. Either sera, renal extracts, or both from SHR were substituted for those from NWR. In this case, the baseline comparison was made with those fragments incubating in the extracts and sera from NWR. In the second series of studies, we followed the effects of sera and extracts singly and combined from SHR, NWR, SD, and WKY. In the latter, the baseline comparison was made with those fragments incubating in medium alone. These two systems have been used previously to study renotropic activity [17].

Results (specific activities and percents) from the in vitro experiments depicted in the tables and figures are derived by averaging values obtained from a minimum of three flasks. Statistics are by group or paired analysis using Student's t test. Statistical significance was set at $P < 0.05$. Some of the results are expressed as the ratio between the specific activities of DNA from the same fragments incubating under the various conditions [17]. When the specific activity of fragments incubating under the test situation (T) exceeds the specific activity of the fragments incubating under the control conditions (C) the ratio was obtained by (T/C − 1), and the number became positive. If the C were greater, the ratio was expressed as a negative number using the formula: 1 − (C/T) [17].

Results

Figure 1 depicts the relationship between the kidney weights and the body weights of 88 SHR and 84 NWR. When comparing the KW/BW of SHR and NWR, no obvious differences were present. The SHR were smaller rats when compared to NWR

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Table 1. Effect of unilateral nephrectomy and sham operation on normal Wistar rats and spontaneously hypertensive rats[a]

Days after uninephrectomy	BW *g*	KW *g*	KW/BW × *100*	KW % *dry*	Total in kidneys: Protein *mg*	RNA *mg*	DNA *mg*	RNA/DNA	Prot/DNA
Normal Wistar rats (NWR)									
0 (12)	158 ± 7.4	0.72 ± 0.03	0.46 ± 0.01	25.6 ± 0.1	88.9 ± 4.7	10.6 ± 1.2	3.42 ± 0.1	3.14 ± 0.50	26.1 ± 1.3
6 (10)	208 ± 7.5	0.91 ± 0.01	0.45 ± 0.01	24.3 ± 0.5	140.6 ± 5.4	11.0 ± 0.3	4.80 ± 0.1	2.30 ± 0.05	29.3 ± 1.4
(10)	183 ± 6.6[b]	1.02 ± 0.04[b]	0.56 ± 0.02[c]	24.0 ± 0.7	149.0 ± 12.4	12.8 ± 1.1	5.03 ± 0.3	2.54 ± 0.07[d]	29.5 ± 0.5
12 (8)	241 ± 13.2	0.91 ± 0.01	0.45 ± 0.01	24.3 ± 0.5	140.6 ± 5.4	11.0 ± 0.3	4.80 ± 0.1	2.30 ± 0.05	29.3 ± 1.4
(8)	215 ± 14.7	1.25 ± 0.07[d]	0.60 ± 0.02[c]	24.3 ± 0.5	175.0 ± 10.7[d]	14.0 ± 0.6[d]	6.76 ± 0.4	2.14 ± 0.21	26.8 ± 1.8
24 (5)	234 ± 14.8	1.00 ± 0.06	0.43 ± 0.01	25.0 ± 0.7	141.7 ± 13.1	12.5 ± 1.4	5.18 ± 0.4	2.43 ± 0.37	27.6 ± 3.2
(5)	206 ± 24.4	1.25 ± 0.16	0.60 ± 0.02[c]	24.7 ± 0.4	188.7 ± 17.1[d]	15.9 ± 2.1	5.94 ± 0.8	2.68 ± 0.14	32.4 ± 2.4
Spontaneously hypertensive rats (SHR)									
0 (12)	145 ± 9.2	0.72 ± 0.05	0.49 ± 0.03	23.1 ± 1.0	108 ± 13.6	9.7 ± 1.4	3.42 ± 0.3	2.79 ± 0.20	31.4 ± 1.5
6 (10)	186 ± 8.0	0.74 ± 0.09	0.45 ± 0.02	20.6 ± 3.3	124.3 ± 8.4	10.0 ± 0.4	4.46 ± 0.3	2.25 ± 0.10	27.7 ± 1.5
(10)	164 ± 6.5[d]	0.94 ± 0.04[d]	0.58 ± 0.02[c]	23.8 ± 0.8	145.7 ± 7.7	12.2 ± 0.3[e]	4.92 ± 0.2	2.48 ± 0.04[d]	29.6 ± 1.0
12 (8)	214 ± 9.5	0.88 ± 0.05	0.41 ± 0.01	24.3 ± 0.2	132.5 ± 10.2	10.9 ± 1.0	5.22 ± 0.5	2.08 ± 0.08	25.4 ± 0.8
(8)	194 ± 13.5	1.02 ± 0.05	0.53 ± 0.02[c]	26.3 ± 1.4	156.7 ± 9.8	13.6 ± 0.8[d]	5.84 ± 0.2	2.34 ± 0.06[b]	26.9 ± 0.9
24 (5)	231 ± 12.8	0.91 ± 0.03	0.40 ± 0.03	24.0 ± 1.0	136.8 ± 11.0	11.8 ± 0.4	4.56 ± 0.5	2.82 ± 0.34	32.9 ± 4.3
(7)	201 ± 10.1	1.25 ± 0.07[e]	0.61 ± 0.03[c]	24.0 ± 0.5	173.4 ± 7.1[b]	15.7 ± 0.6[c]	6.23 ± 0.5[d]	2.63 ± 0.18	28.8 ± 2.6

Abbreviations: BW, body weight; KW, kidney weight; % dry, percentage of total kidney weight which was dry mass; ( ), represents number of rats studied.

[a] Top value under each heading represents data from sham-operated rats; below it is value from rats following unilateral nephrectomy.

[b] $P < 0.02$

[c] $P < 0.001$

[d] $P < 0.05$

[e] $P < 0.01$ compared to sham-operated rats studied at same time interval (upper value).

rats of the same age. Therefore, their body weights tend to be on the left side of the figure and rats with body weights above 350 g were mainly NWR. With few exceptions, the kidney weight/body weight in those rats under 250 g in size are comparable among the two groups. Thus, the size of kidneys in SHR is not unusual.

Figure 2 depicts the relative differences in specific activity of DNA when renal slices incubated in ^{3}H-thymidine for 90 min. Slices were obtained from age-matched SHR and NWR and analyzed simultaneously. NWR were made controls. Up to 15 weeks of age, the incorporation of ^{3}H-thymidine into DNA, a rough estimate of DNA synthesis which depends a great deal on the size of precursor pools, is not particularly different. Perhaps, SHR have a slightly greater incorporation at 25 weeks of age.

Table 1 shows the effect of unilateral nephrectomy and sham-operation on normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR). In both groups, there was a tendency for rats to gain less weight by day 6 postunilateral nephrectomy compared to sham-operation. However, the rate of weight gain is similar in both groups after this time period. The kidney weight (KW) of the remaining kidney is greater 6 days postoperation in both substrains when compared to sham-operated rats and significantly different in the normal Wistar rats 12 days after operation. By 24 days after operation the weight of the single kidney remained greater in the SHR but was not greater statistically in normal Wistar rats due to the great variation in weights. Within the latter group, one of the rats lost a great deal of body weight which may have contributed to poor renal growth. When one calculates the kidney weight/body weight ratio, it is seen that from 6 days postunilateral nephrectomy there was a significant increase in this ratio in NWR and SHR. The percent of kidney weight that is dry mass was consistently the same throughout the study in both groups. While the total RNA, protein, and DNA in the remaining kidneys of both groups tended to be larger 6 days after unilateral nephrectomy, there was no uniformed statistical significance due to the large variation in values. However, there was a rise in the RNA/DNA ratio at 6 days postunilateral nephrectomy in both groups and at 12 days postoperation in SHR. These results show the expected findings in the normal Wistar rats, that is, an increase in kidney size after unilateral nephrectomy as well as a tendency for an increase in total protein RNA, DNA, and RNA/DNA [24]. The added finding is that the same characteristics also occur in SHR. From our results, we conclude that there are no apparent major differences in the renal response of SHR and NWR following unilateral nephrectomy.

Figure 3 depicts the in vitro data derived from the materials of 6 SHR that had received a unilateral nephrectomy compared to 6 SHR that had received only sham-operations. The serum and renal extract from unilaterally nephrectomized SHR were compared to renal fragments (obtained from SD) incubating only in serum and renal extract from sham-operated SHR. These

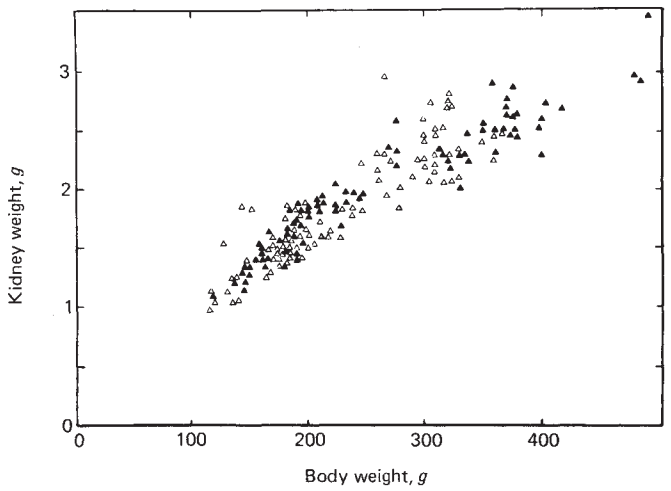

Fig. 1. *Correlations between the body and kidney weights of 172 rats.* Approximately one-half were normal Wistar rats (NWR, ▲) and one-half were spontaneously hypertensive rats (SHR, △).

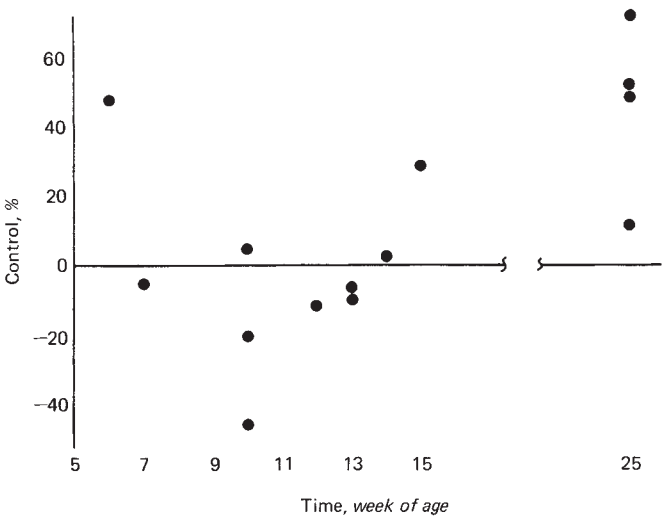

Fig. 2. *The difference in uptake of ^{3}H-thymidine by renal fragments obtained from age-matched spontaneously hypertensive (SHR) and normal Wistar rats (NWR).* The percentage of specific activity of the SHR fragment DNA (average value from three flasks) is compared to the DNA specific activity of renal fragments from control NWR (average value from three flasks). See the **Methods** section for details.

studies were similar to ones performed originally on Sprague-Dawley rats [14, 16–18, 20]. They show that when sera from unilaterally nephrectomized rats (uni) replaced sera from sham-operated rats (sham), when uni renal extracts replaced sham extracts and when both uni sera and extracts replace sham sera and extracts that there are statistically significant increases in the incorporation of ^{3}H-thymidine into the renal DNA of incubating fragments obtained from control Sprague-Dawley rat kidneys. While slightly higher in stimulation, these results resemble and corroborate those reported when the same studies were performed in renal extracts and sera from Sprague-Dawley rats [17].

Table 2 depicts data from 18 studies which were similar to those shown in Figure 3. The exception was that the comparisons were made between extracts and sera obtained from normal NWR and SHR who received no operations. In this case, the various products obtained from SHR rats are compared to the effects of the products from the NWR rats. The baseline for comparison derives from those renal fragments from Sprague-Dawley rats that are incubating in renal extracts and sera from normotensive Wistar rats (NWR). When sera from the SHR rats were substituted for that obtained from NWR rats, there is a statistically significant increase in the specific activity of renal DNA in incubating fragments. The overall increase is 13.7% ± 3.7 (SEM) ($P < 0.01$). When SHR renal extracts replaced the extracts from the NWR, the increase in renal DNA of 8.8% ± 5.5 (SEM) (P = NS) did not prove to be statistically significantly different. Substitution of both renal extracts and sera from SHR caused the greatest relative stimulation of all, 20.6% ± 6.2 (SEM) ($P < 0.01$). Thus, the only significant stimulations to the isotopic incorporation of ^{3}H-thymidine into DNA was seen when sera from the SHR rats replaced sera from the NWR rats. Extracts from SHR did little until sera from SHR was substituted into the system.

The results depicted in Tables 3 and 4 were experiments performed on sera and extracts from normotensive and hypertensive rats. In Table 3 we see the results when the sera and extracts were obtained from three different strains of normotensive rats (SD, NWR, and WKY) in 23 experiments. Since results using the products from SD, NWR, and WKY were similar, these are grouped together for statistical evaluation. Comparing renal fragments from SD incubating in sera, to those SD fragments incubating in medium alone, it can be seen that there is no significant increase in specific activity of DNA. This is true also when fragments incubating in renal extracts alone and renal extracts plus sera are compared to those renal fragments incubating in medium alone. In contrast, those results depicted in Table 4 using products from SHR show a different influence of serum on isotope incorporation into DNA. When SHR sera were added to the incubation medium in 18 experiments, there was a 12.7% ± 4.7 (SEM) increase in incorporation of isotope. Whether the statistics were performed by comparing the specific activities or the differences in the percent stimulation from 0, it was found to be statistically significant ($P < 0.01$ and $P < 0.02$, respectively). In contrast, addition of extracts from SHR alone caused no change in the specific activities of renal fragments compared to control. A combination of sera and extracts from SHR showed no statistically significant increase in isotope incorporation, although there was some elevation.

When perusing the data depicted in Tables 2 and 4, it appeared to us that the least stimulation by sera from SHR was seen when the rats were older, that is, when they exceeded 19 weeks of age. To determine this, we depicted the overall data in a temporal fashion in Figures 4 and 5. Figure 4 illustrates the data obtained from Table 3, that is, it compared the effects of sera obtained from normotensive rats. It shows the percentage of stimulation or depression when the fragments incubating in "normotensive sera" are compared to those incubating in medium alone and also when those fragments incubating in sera plus extract are compared to those incubating in extract alone.

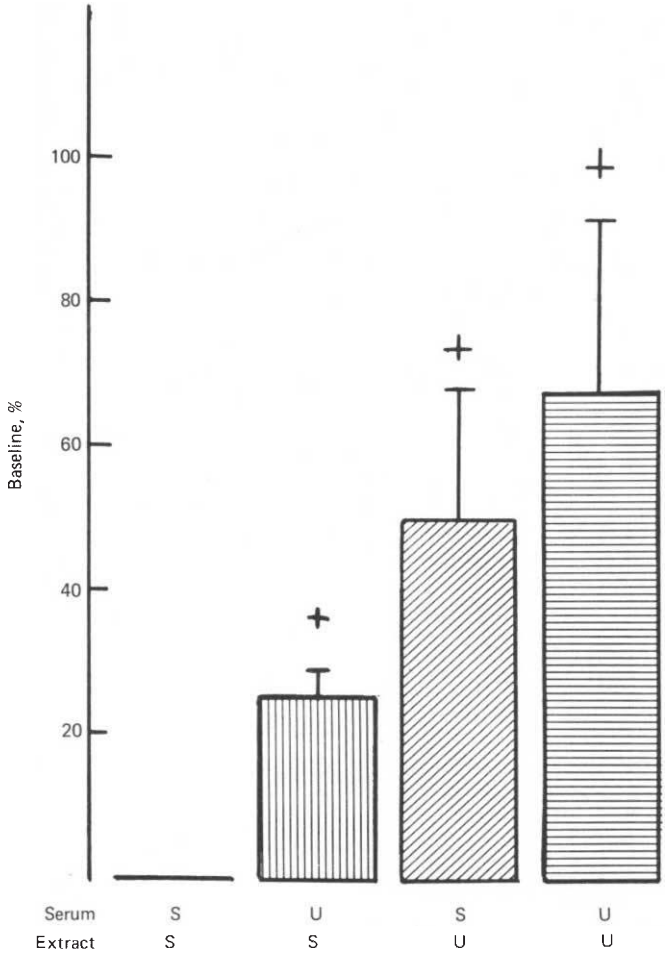

Fig. 3. *The ability of serum, renal extracts, and serum plus extracts from unilaterally nephrectomized SHR* (U) *to stimulate* ^{3}*H-thymidine into DNA of incubating renal fragments from Sprague-Dawley rats when compared to these products from sham-operated SHR* (S). Average ± SEM of six experiments is depicted. Baseline for comparison as indicated in the figure is the situation in which sera and extracts come from sham-operated rats. Symbol: +, $P < 0.05$ compared to baseline.

In other words, we compare the stimulation or lack of stimulation when the only variable between flasks becomes the addition of serum. It can be seen (Fig. 4) that no matter what the age of the rat from which the products are obtained, the serum has no stimulatory effect on the incorporation of ^{3}H-thymidine into DNA.

This can be contrasted with the data depicted in Figure 5 where the sera originates from SHR. In this case, when we display the comparisons from Tables 2 and 4 where SHR sera is the variable in the flasks, it can be seen that the majority of the results show stimulation up to 16 weeks of age. In the 72 paired flasks where sera from SHR rats is the variable, the overall increase is 11.1% ± 1.7 (SEM) ($P < 0.001$). The increase is even greater when only those rats 16 weeks of age and under are considered. In this case, there are 48 paired flasks which show an overall stimulation of 13.5% ± 2.1 (SEM) ($P < 0.001$). In the rats that are 19 weeks of age or greater, the overall stimulation is 6.3% ± 3.3 (SEM). The latter misses statistical significance ($P > 0.05$). Between 19 to 21 weeks of age, many SHR do not display measurable renotropic activity in their sera.

Discussion

In the 1950's, Braun Menendez [1, 2] suggested that circulating factors, which he named renotropins, control renal growth. Because renal growth occurs in many experimental situations that produce hypertension, Braun Menendez further postulated that increased concentrations of renotropins not only initiated and regulated renal growth but formed the basis for hypertension. His hypothesis states that hypertension develops when the remaining renal tissue is unable to respond to renotropins which, in turn, increase the concentrations of renotropins. Through some unexplained mechanisms, renotropins influence blood pressure.

In support, Greenwood, Nassim, and Taylor [25] noted that normal kidneys had to be removed to maintain Goldblatt hypertension. They proposed that "either the normal kidney excretes or destroys a renal pressor substance or that the increased work load imposed on the ischemic kidney results in an increased production of a pressor substance." Later, Fregly and Field [26] noted a correlation between kidney size and hypertension, that is, the greater the kidney weight, the higher the blood pressure. Braun Menendez [1, 2] felt that the weakest link in his hypothesis was that the existence of renotropin was unproven. Over recent years, various studies on unilaterally nephrectomized rats have demonstrated that renotropins are present in blood and urine [3–21].

Remaining viable kidney tissue grows by hypertrophy (cell enlargement) and hyperplasia (cell division) [24]. After unilateral nephrectomy, RNA and DNA synthesis increase early [27, 28], preceding the onset of obvious compensatory growth. The "work hypothesis" as a major factor in CRG appears not to be viable, because unilateral ureteroduodenostomy without nephrectomy does not cause CRG in the contralateral kidney [29] even though only one kidney acts as an effective excretory organ. Also, unilateral ureteroperitoneal fistula neither enhances renal DNA synthesis [30] nor mitotic rate [31] in the same manner as unilateral nephrectomy.

Three types of experiments have been performed to determine the presence of renotropins. These include parabiotic, in vivo serum injections, and in vitro experiments. In general, the three different methodologies support the existence of renotropins. Convincing evidence is provided by parabiotic experiments [3–10] utilizing vascular cross-circulation techniques. Van Vroonhoven, Soler-Montesinos, and Malt [5] demonstrated that a vascular connection between an anephric rat and a normal partner produced marked compensatory renal hypertrophy in the partner, resembling that seen in the remaining kidney of a single rat following unilateral nephrectomy. They suggested that these data support the renotropin hypothesis and lessen the importance of the neural and circulatory-mechanical theories of CRG. The renotropic activity of sera from unilaterally and bilaterally nephrectomized animals has been investigated by injecting sera into normal animals. Lowenstein and Stern [11] and others [12, 13] using multiple intraperitoneal injections demonstrated that sera from rats 48 hr after unilateral nephrectomy increased ^{3}H-thymidine uptake in kidney cell nuclei of intact rats. However, not all injection studies have been quite successful [32–34].

Table 2. Average specific activity of fragments from Sprague-Dawley rats incubating in various combinations of sera and renal extracts from SHR and NWR[a]

Experiment no.	Age *weeks*	$W_k + W_s$ SA	$W_k + SHR_s$ SA	%	$SHR_k + W_s$ SA	%	$SHR_k + SHR_s$ SA	%
1	9	75.9	84.9	(+12)	72.9	(−4)	72.4	(−5)
2	9	205.4	220.2	(+7)	172.7	(−19)	221.3	(+8)
3	12	75.3	85.1	(+13)	121.1	(+61)	130.0	(+73)
4	13	73.2	92.7	(+27)	80.0	(+9)	98.6	(+35)
5	14	124.4	158.7	(+27)	115.8	(−7)	114.8	(−8)
6	15	92.4	101.6	(+10)	104.7	(+13)	107.9	(+17)
7	16	64.6	83.1	(+29)	71.9	(+11)	84.9	(+31)
8	16	107.8	128.7	(+19)	120.4	(+12)	124.7	(+16)
9	6	205.2	193.8	(−6)	186.9	(−10)	215.9	(+5)
10	7	168.6	155.8	(−8)	137.5	(−22)	161.7	(−4)
11	8	190.3	212.4	(+11)	171.6	(−11)	215.8	(+13)
12	9	101.5	142.9	(+41)	142.2	(+40)	139.1	(+37)
13	10	264.2	264.1	(0)	256.0	(−4)	291.6	(+10)
14	19	79.2	86.5	(+9)	90.0	(+14)	97.0	(+22)
15	20	75.5	75.1	(0)	84.7	(+12)	88.9	(+18)
16	20	36.6	40.9	(+12)	38.6	(+5)	49.3	(+35)
17	21	111.5	108.8	(−2)	98.1	(−13)	87.9	(−13)
18	21	52.8	76.5	(+45)	79.5	(+50)	95.7	(+81)
Average		116.9	128.4	13.7	119.1	7.6	133.2	20.6
SEM		15.4	14.8	3.7	12.7	5.6	15.5	6.2
P			<0.01	<0.01	NS	NS	<0.01	<0.01

Abbreviations: SHR, spontaneously hypertensive rats; NWR, normal Wistar rats; W_k, kidney extract from NWR; SHR_k, kidney extract from spontaneously hypertensive rats; W_s, sera from NWR; SHR_s, sera from spontaneously hypertensive rats; SA, specific activity; %, percent stimulation (+) or depression (−) compared to ($W_k + W_s$).

[a] Each value represents the average specific activity from three flasks.

[b] *P* values were derived from Student's *t* test and paired analysis. For %, *P* values indicate whether or not the value is significantly different from 0.

A series of studies by Preuss, Terryi, and Keller [14], Preuss and Goldin [16, 17, 19], Preuss, Goldin, and Shivers [18], and Castillo et al [20] provides additional evidence to support the existence of renotropins. In 1970 Preuss, Terryi, and Keller [14] described an in vitro assay for renotropin consisting of rat kidney tissue incubating in Krebs-Ringer's bicarbonate solution containing ^{3}H-thymidine monophosphate and ^{14}C-uridine. The incorporation of these isotopes into DNA and RNA was used as indicators of synthesis. Various procedures were carried out to minimize differences in intracellular precursor pools for DNA and RNA synthesis. Compared to plasma from sham-operated rats, plasma from unilaterally nephrectomized (uni) rats (18 to 24 hr postoperation) significantly augmented the incorporation of ^{3}H-thymidine and ^{14}C-uridine into DNA and RNA of incubating tissue [14, 16–20]. The uni plasma was relatively specific for renal cortices, as isotopic incorporation into slices of rat kidney medulla, liver, spleen, and lung was not enhanced by uni plasma [14, 16]. Since uni plasma did not stimulate renal medullary RNA and DNA synthesis, the growth factor described here may be different from that described earlier by Ogawa and Nowinski [35]. Lyons et al [15] also showed that uni sera caused a relative increase of uridine into RNA in tissue culture over a matter of hours. Later, Castillo et al [20] showed by autoradiography that uni sera stimulated renal DNA synthesis in vitro and Dicker and Morris [21] found that uni sera could cause a relative increase in the weight and protein content of tissue slices incubating for as short a period as 2 hr.

Because we have been unable to find a renotropic factor in the circulation of unilaterally nephrectomized rats [14, 16–20] and because renotropin and renal growth have been associated with hypertension [1, 2, 26], we undertook a series of studies to discern renotropic activity and renal growth in spontaneously hypertensive rats.

In recent works, Okamoto and Aoki [36] and Okamoto [37] described the development of a strain of Wistar rats which become hypertensive spontaneously around 8 weeks of age. The most rapid rise in blood pressure occurs between weeks 8 and 13 of life. The genetic background and pathology of the rats suggest that they may be the ideal laboratory model to study human essential hypertension.

While others have shown an association between large kidneys and hypertension [26], we could find no enlargement of the SHR kidneys when compared to normal (Fig. 1), which was in agreement with others [38]. When we compared the renal growth characteristics of SHR and NWR following unilateral nephrectomy, there were no obvious differences in the kidney weight/body weight, kidney dry weights, total RNA, total DNA, total protein, RNA/DNA, and protein/DNA. Both groups of rats responded in a similar expected way to removal of a single kidney [24]. Sera and renal extracts removed 24 hr after unilateral nephrectomy in SHR-stimulated ^{3}H-thymidine incorporation into renal DNA of incubating control renal fragments relatively more than the sera and renal extracts from sham-operated SHR. Comparing these results with those found in normotensive Sprague-Dawley rats, the stimulation may even be slightly greater [17]. Admittedly, this must be investigated further; however, this suggested to us that renotropic activity may be greater in SHR to maintain renal size and DNA

Table 3. Average specific activities of fragments from Sprague-Dawley rats incubating in sera, renal extracts, and sera plus renal extracts from different strains of normotensive rats

Experiment no.	Age *weeks*	Control SA	Serum SA	Serum %	Extract SA	Extract %	Extract + Serum SA	Extract + Serum %
SD								
1	10	59.0	59.6	(+1)	55.8	(−6)	50.2	(−17)
2	12	62.7	65.6	(+4)	61.8	(−1)	71.2	(+13)
3	14	149.2	128.5	(−16)	135.1	(−10)	127.1	(−17)
NWR								
4	13	79.0	89.6	(+13)	71.2	(−11)	87.2	(+10)
5	10	37.9	38.7	(+2)	36.6	(−4)	40.0	(+5)
6	10	84.2	77.9	(−8)	72.2	(−17)	77.6	(−8)
7	11	82.9	76.4	(−8)	78.1	(−6)	81.1	(−2)
8	11	75.3	81.1	(+8)	65.8	(−14)	119.2	(+58)
WKY								
9	8	84.2	77.9	(−8)	72.2	(−17)	77.6	(−8)
10	8	82.9	76.4	(−8)	78.1	(−6)	81.1	(−2)
11	6	83.2	78.2	(−6)	89.4	(+7)	73.8	(−13)
12	7	102.7	106.1	(+3)	103.6	(+1)	94.0	(−9)
13	8	105.2	104.1	(−1)	109.4	(+4)	106.0	(+1)
14	9	205.7	190.3	(−8)	198.6	(−3)	188.7	(−9)
15	10	57.4	59.6	(+4)	52.4	(−9)	54.0	(−6)
16	11	51.0	49.6	(−3)	45.6	(−12)	41.8	(−22)
17	12	101.6	99.3	(−2)	84.5	(−20)	83.0	(−22)
18	13	110.7	111.9	(+1)	105.6	(−5)	96.3	(−15)
19	14	109.7	115.5	(+5)	115.5	(+5)	110.1	(0)
20	14	64.4	61.9	(−4)	63.5	(−1)	61.1	(−5)
21	15	58.2	57.4	(−1)	54.9	(−6)	55.1	(−6)
22	16	66.4	61.9	(−7)	64.9	(−2)	60.8	(−9)
23	17	49.7	40.1	(−24)	41.8	(−19)	37.9	(−31)
Average		85.4	82.9	−2.7	80.7	−6.4	81.5	−5.0
SEM		7.8	7.2	1.7	7.6	1.6	7.3	3.7
P			(NS)	(NS)	<0.01	<0.001	(NS)	(NS)

Abbreviations and symbols: Control, fragments incubating in medium alone; SA, specific activity of renal DNA; %, percent of stimulation (+) or depression (−) compared to control.

[a] Each value represents the average specific activity from three flasks.

[b] *P* values were derived from Student's *t* test and paired analysis. For %, *P* values indicate whether or not the value is significantly different from 0.

Table 4. Average specific activities of fragments from Sprague-Dawley rats incubating in sera, renal extracts, and sera plus renal extracts from spontaneously hypertensive rats (SHR)

Experiment no.	Age *weeks*	Control SA	Serum SA	Serum %	Extract SA	Extract %	Extract + Serum SA	Extract + Serum %
1	19	238.2	256.7	(+8)	225.7	(−5)	222.6	(−7)
2	19	156.2	143.8	(−8)	162.7	(+4)	162.9	(+4)
3	19	136.6	113.4	(−20)	122.3	(−11)	115.3	(−18)
4	19	93.3	80.9	(−3)	91.5	(−2)	83.9	(−11)
5	13	206.3	215.6	(+4)	200.3	(−3)	189.4	(−9)
6	13	258.2	303.0	(+17)	245.0	(−5)	284.9	(+10)
7	13	148.5	176.6	(+19)	166.0	(+12)	180.6	(+22)
8	15	235.7	240.4	(+2)	219.2	(−7)	224.4	(−5)
9	15	74.2	100.2	(+35)	82.2	(+11)	95.4	(+28)
10	15	291.0	324.6	(+29)	296.3	(+2)	355.2	(+22)
11	13	52.4	86.9	(+66)	50.0	(−5)	62.9	(+20)
12	13	171.8	181.9	(+6)	173.6	(+1)	180.2	(+5)
13	13	61.3	72.3	(+18)	65.4	(+6)	65.7	(+7)
14	14	163.7	184.3	(+12)	157.3	(−4)	161.1	(−2)
15	14	86.2	104.2	(+21)	96.1	(+11)	89.9	(+4)
16	20	58.6	72.6	(+24)	58.7	(0)	78.3	(+34)
17	20	52.7	48.6	(−8)	46.7	(−13)	57.4	(+10)
18	20	49.1	52.3	(+6)	45.5	(−8)	43.5	(−13)
Average		140.8	153.2	12.7	139.1	−0.9	147.4	5.6
SEM		19.3	21.0	4.7	18.7	1.8	20.9	3.6
P			<0.01	<0.02	(NS)	(NS)	(NS)	(NS)

See Table 3 for abbreviation and symbol meanings and footnotes.

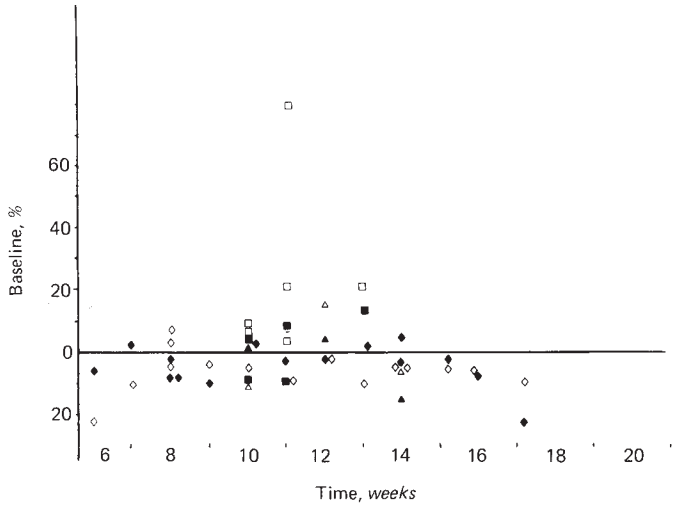

Fig. 4. *The ability of sera from untouched Sprague-Dawley rats* △, NWR ■, and WKY ◆ *of different ages to enhance the incorporation of* ^{3}*H-thymidine into the DNA of incubating renal fragments from SD.* Baselines for comparison are the renal fragments incubating in medium alone or renal extracts alone. *Solid figures* are comparisons between columns 1 and 2 and *open figures* are comparisons between columns 3 and 4 in Table 3.

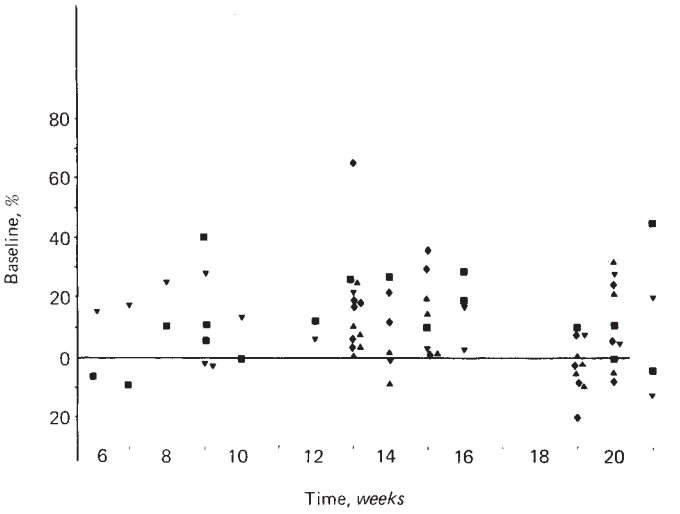

Fig. 5. *The ability of sera from untouched SHR of different ages to enhance the incorporation of* ^{3}*H-thymidine into the DNA of incubating renal fragments from Sprague-Dawley rats.* Comparisons are columns 1 and 2 in Table 4 (◆), columns 3 and 4 in Table 4 (▲), columns 1 and 2 in Table 2 (■), and columns 3 and 4 in Table 2 (▼).

synthesis to the same degree as in unilaterally nephrectomized normotensive rats.

Preuss and Goldin gathered evidence for a renal tissue factor contributing to enhanced DNA [17] and RNA [19] synthesis following unilateral nephrectomy. A tissue extract from kidneys 20 hr after the removal of the contralateral kidney, in the presence of even sham serum, stimulates isotopic incorporation into DNA and RNA in their in vitro assay. A possibility is that the tissue factor is necessary to activate the humoral one, because addition of both uni components to liver slices can increase ^{3}H-thymidine incorporation into liver slices whereas uni sera alone cannot [39]. This same factor can be assayed in SHR following unilateral nephrectomy (Fig. 3) but cannot be assayed in renal tissue from untouched SHR.

Although the renal size of SHR and their response to unilateral nephrectomy is not greatly different from normotensive rats, sera from control untouched SHR showed relatively more renotropic activity than that obtained from three strains of normotensive rats (Table 2 to 4; Figs. 4 and 5). The activity of this factor seems to be greater in younger SHR (6 to 16 weeks) and may decrease beyond 19 weeks of age. Thus, it is measurable during the period of time when there is the greatest acceleration in the blood pressure rise.

To summarize, we are able to show a relative increase in renotropic activity in the serum of untouched SHR but not NWR, SD, and WKY. Activity was found greatest at 6 to 16 weeks of age, a time when the level of blood pressure is rising rapidly. Because kidneys of SHR are normal in size, it is suggested that higher renotropic activity somehow balances with kidney size in SHR, that is, they have a higher concentration to maintain normal renal size. As a final point, unilateral nephrectomy in SHR is associated with higher blood pressures [40].

Our findings are comparable with the Braun Menendez hypothesis [1, 2]. Obviously, more work will have to be performed to determine the physiological roles of renotropins other than the control of renal growth [41]. It is possible that renotropins are elevated in response to some subtle renal damage secondary to the elevated blood pressure in SHR. We cannot dispute this; however, we and others have not been able to find obvious renal damage or even renal dysfunction in this age group of SHR [22, 37, 38].

Summary. Studies performed in the 1950's suggested that a circulating factor controlling renal growth (renotropin) could contribute to hypertension. However, no assay was available to prove its existence. Recently, different assays have been able to demonstrate the presence of a circulating renotropic factor following unilateral nephrectomy in rats. Therefore, we investigated certain aspects of renal growth in SHR, especially serum renotropic activity, and compared these with the same parameters in three strains of normotensive rats (SD, NWR, and WKY). Renal slice and renal DNA synthesis in response to unilateral nephrectomy were not unusual in SHR compared to other strains previously studied. Sera and renal extracts from young SHR following unilateral nephrectomy compared to sera and renal extracts from sham-operated SHR stimulated ^{3}H-thymidine incorporation into the DNA of renal fragments. This pattern was similar to findings when sera and renal extracts from unilaterally nephrectomized SD were investigated, but the sera and extracts from SHR may have shown greater overall stimulation. Interestingly, a relative increase in renotropic activity was found in the serum of untouched SHR (11.1% ± 1.7 (SEM), $P < 0.001$) but not untouched NWR, SD, and WKY. The greatest renotropic activity in SHR was found at 6 to 16 weeks of age (13.5% ± 2.1 (SEM), $P < 0.001$). The previously reported activator found in renal tissue after unilateral nephrectomy was not found to be increased in untouched SHR. No studies were performed on SHR greater than 25 weeks of age. As a first

approximation, our investigations are consistent with a previously proposed hypothesis that renotropin may play some role in hypertension.

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